REMARKS

Claims 1-5 are pending in this application. Claim 1 has been amended. Support for the amendment to Claim 1 may be found generally throughout the specification, including paragraphs 27-28. No new matter has been added by virtue of this amendment.

The Examiner relies on five references in combination in his attempt to show obviousness of the pending claims, despite acknowledged differences in the primary reference Oldenburg. The Examiner basically ignores these differences by alleging that the other four references address the shortcomings of Oldenburg. Applicants respectfully traverse, as the Examiner is mistaken and ignores the teaching of Oldenburg, which contrary to the Examiner's interpretation, teaches away from the method of Claims 1-5.

A. Summary of Oldenburg

Oldenburg discloses a specific method for expression and purification of a PTH analog, especially "when combined with purification by nickel chelation chromatography and subsequent cleavage of the polymer into monomeric units (...)". Oldenburg acknowledges this method for producing certain peptides which "will also contain a homoserine/homoserine lactone residue at the carboxyl-terminal" (page 279, right column, last paragraph before the Materials and Methods section). Additional detailed method steps are disclosed by Oldenburg on page 281 (bridging paragraphs):

- Collection of the inclusion bodies
- Washing with 50 mM Tris-H Cl, pH 7.5, 10 mM EDTA, 100 mM KCl
- Washing with 10 mM Tris-HCl, pH 7.5
- Resuspension of the pellet in 10% SDS
- Dilution of the SDS concentration to 1% by the addition of a solution comprising 5 mM imidazole, 500 mM NaCl, 20 mM Tris-HCl, pH 7.9
- Loading to a his-bind column.

B. Differences of Oldenburg and Claim 1

As adduced previously in Applicants response of March 6, 2009, **Oldenburg fails to mention, teach, nor suggest at least four claimed elements of Claim 1**:

1) No additional modification step is needed to obtain the unmodified protein (Oldenburg in contrast would, at the very least, require an additional modification step to eliminate the homoserine or homoserine lactone caused by its procedure);

2) A washing step of at or below pH 6.5 (i.e. acidic pH) (Oldenburg in direct contrast requires a basic pH of 7.5, a magnitude of at least ten-fold (10) difference in pH and a complete difference in kind)[1];

3) A solubilizing step in the absence or detergents or denaturing agents (Oldenburg in direct contrast requires detergent to solubilize, which would affect the nature of the peptide by definition); and

4) A resultant antifusogenic peptide with a glycine at its C-terminus (Oldenburg, as acknowledged by the Examiner, does not even teach nor suggest antifusogenic peptides; Oldenburg's method further results in the presence of the homoserine/homoserine lactone at the carboxyl terminus).

C. Teaching Away of Oldenburg

In addition to the differences noted above, Oldenburg's method inherently and distinctly teaches away from the method of Claim 1 in at least three distinct regards: 1) requiring metal chelation chromatography, 2) requiring cleaving the peptide with CNBr, and 3) the resultant peptide containing a potentially hindering homoserine/homoserine lactone residue at the carboxyl-terminal.

[1] The Examiner contends that modifying pH levels is akin to modifying temperature or concentration levels to an optimum level and thus would generally not be considered patentable as such would be a "general condition() of a claim". However, Applicants respectfully point out the pH level taught by Oldenburg is basic, whereas Applicants claimed pH level is acidic. One of ordinary skill in the art would not generally modify or optimize a taught basic pH outside of the range of a basic pH – i.e. change the basic pH to an acidic pH. As Applicants' specification notes [0047], acidic denaturing conditions were found surprisingly not to solubilize the fusion polypeptide to a considerable extent (but did solubilize a lot of other impurities).

The Examiner has consistently failed to address that Oldenburg's method results in a peptide which also contains a homoserine/homoserine lactone residue at the carboxyl-terminal. In other words, Oldenburg's method teaches a clear, explicit and unambiguous warning that its method will only work for producing polypeptides whose biological activity is not changed by a homoserine at the C-terminus.

The Examiner is silent on Oldenburg's teaching of the homoserine/homoserine lactone residue at the carboxyl-terminal. The Examiner has never addressed how it would be obvious to use the method of Oldenburg to obtain polypeptides whose biological activity is changed or affected by a homoserine/homoserine lactone at the carboxy-terminal. Respectfully stated, the Examiner has not met his burden of showing obviousness in light of Oldenburg's method requiring such a residue at the carboxy-terminal.

D. Failure of Combined References to Address/Remedy Acknowledged Teaching away and Differences of Oldenburg from Claim 1

As Oldenburg fails to teach or suggest at least the four noted differences in section A) above, and teaches away from the method of Claim 1 as noted in section C) above, the additional four cited references must resolve these differences and reverse the teaching away deficiencies in order to support a proper, prima facie 103(a) rejection. However, and contrary to the Examiner's repeated allegations, the combination of Vandenbark, Lambert, McCoy and Mukhopadhyay do not remedy all the differences, nor does the combination address, much less reverse, the teaching away deficiencies of Oldenburg. For this reason, Applicants respectfully submit that the 103(a) rejection must be withdrawn.

Presuming arguendo for the sake of this section a motivation to combine said references with Oldenburg (see Section E) below), the combination of Vandenbark, Lambert, McCoy and Mukhopadhyay do not address all the differences and defiencies of Oldenburg's method as compared with Claim 1.

1) Mukhopadhyay

First, Mukhopadhyay et al, a general textbook, points out in section 2.2.3 that "Solubilization of these aggregates requires disruption of the forces that hold them together, by using chaotropic agents or detergents" (" Chaotropic agents, such as GuHCl and urea, are commonly used for the solubilization of inclusion bodies", page 86, beginning of 2nd paragraph). In Chapter 4,4, 2nd line, Mukhopadhyay reports that "Aggregated prteins are solubilized in 6 mol/GuHCl or in 8 mol/l urea". "Initially, inclusion body is dissolved in strong denaturant (6mol/l GuHCl or in 8 mol/l urea)" as outlined in 5.3.2.2. 3rd to 5th line.

Thus, Mukhopadhyay[2], like Oldenburg et al., is also completely silent about a washing step at a pH value below 6.5 in the presence of a denaturing agent followed by a solubilization step at a pH above 9.0 and in the absence of a denaturing agent.

2) McCoy

Second, the addition of McCoy to the combination of Mukhopadhyay with Oldenburg similarly does not remedy any of the differences and deficiencies of Oldenburg. McCoy et al. reports a method comprising a washing step with pure water and a solubilizing step at a pH of over 12 (Example 1 and 2).

Thus, as as Oldenburg et al. and Mukhopadhyay is also McCoy et al. completely silent about a combination of a washing step in the presence of denaturing agents and a solubilization step in the absence of denaturing agents.

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[2] Indeed, with regard to the use of detergents, Mukohopadhyay teaches away from Applicants' claimed method. Indeed, Mukohopadhyay affirmatively teaches that use of detergents for the solubilization of inclusion bodies is common in the art and that one of ordinary skill in the art would be motivated to use detergents for the solubilization of inclusion bodies:

> "Detergents of any form (cationic, anionic and zwitterionic) have been used for the solubilization of inclusion bodies. [...] Thus, detergent just masks certain hydrophobic patches on the protein surface and enhances its solubility." (p. 86)

Thus, the addition of Mukohopadhyay to Oldenburg actually supports (and encourages one of ordinary skill in the art to use) the Oldenburg method of using detergents to solubilize!

3) Lambert

The addition of Lambert, which is alleged to disclose a number of antifusogenic peptides, to the combination of McCoy, Mukhopadhyay and Oldenburg likewise does not remedy any of the differences and deficiencies of Oldenburg. Lambert, like McCoy, Oldenburg et al. and Mukhopadhyay is also completely silent about a combination of a washing step in the presence of denaturing agents and a solubilization step in the absence of denaturing agents.

4) Vandenbark

The addition of Vandenbark, a non-inclusion body reference allegedly cited to disclose attachment of a C-terminal glycine residue, to the combination of McCoy, Mukhopadhyay and Oldenburg likewise does not remedy any of the differences and deficiencies of Oldenburg. Lambert, like McCoy, Oldenburg et al. and Mukhopadhyay is also completely silent about a combination of a washing step in the presence of denaturing agents and a solubilization step in the absence of denaturing agents.

Additionally, one of ordinary skill in the art would not have even considered Vandenbark in light of the methodology of Oldenburg. Oldenburg limits[3] the applicability of its procedure with regard to other proteins:

[t]**his method is restricted**, of course, to those peptides which are tolerant of the deletion or replacement of methionine residues. In addition, the peptide will also contain a homoserine or homoserine lactone at the carboxyl-terminus which…may also **compromise {the peptide's} bioactivity** and may pose difficulties if a free carboxyl terminus is needed for peptide activity". Pg 283 (emphasis added)

[3] Additionally, as part of its acknowledged limited and restricted method, Oldenburg requires washing the isolation bodies at ph 7.5 and with 100 ml of 10mM Tris-HCl with 100ml of WTEK (100mM KCl), then resuspension in 10 ml of 10% SDS (a detergent) to solubilize and even sonification of the sample "was necessary to solubilize all of the protein". Binding buffer is then added. (See page 281).

Thus, Oldenburg acknowledges the restriction of its process to certain proteins, acknowledges the presence of a homoserine/homoserine lactone which may compromise the bioactivity of the protein and thus would require an additional modification step to obtain the unmodified protein, requires that the washing step at a pH of 7.5, and the finally additionally requires re-suspension in a detergent.

Thus, why would one of ordinary skill in the art looked at and use an non-inclusion body art reference (Vandenbark) to somehow attach a C-terminal glycine residue, given the explicit warning of Oldenburg limiting its procedure to certain peptides (including those involving the carboxyl terminus)? This question, which underlies motivation to combine, has not been even considered, much less addressed, by the Examiner.

Finally, none of the cited references, neither singularly nor in combination, teach how to reverse the teaching away of Oldenburg regarding the resulting homoserine/homoserine lactone. No cited reference ever addresses this issue, which Oldenburg explicitly acknowledges may compromise the biochemistry of the resulting protein and thus requires an additional modification step. Since no reference, neither nor in combination, addresses/remedies the acknowledged deficiencies and limitations of Oldenburg, the combination of the cited art also fails to address/remedy said deficiencies and self-acknowledged limitations of Oldenburg.

E. Lack of Motivation to Combine Said References with Oldenburg

Applicants additionally note that there is no motivation to combine the cited references, especially Vandenbark and Lambert, with Oldenburg. The Examiner has not shown why a person of ordinary skill in the art would be motivated to combine Vandenbark (alleged C-terminal glycine residues) and Lambert (alleged antifusogenic HIV-1 polypeptides) within the actual process method of Oldenburg. Oldenburg **explicitly warns** against using its method for producing a peptide which would be affected by a homoserine/homoserine lactone residue. Far from being prima facie obvious, the utilization of Oldenburg's method, which requires such harsh techniques as nickel chelation chromatography and cyanide bromide cleavage of peptides and results in a potentially hindering homoserine/homoserine lactone residue, would NOT be obvious to one of ordinary skilled in the art looking to produce a peptide that would not be active (or less active) with a lactone residue.

Thus, Applicants respectfully submit that even if, *arguendo*, the combination of said references remedies the acknowledged differences and teaching away of Oldenburg, that one of ordinary skill in the art would not have been motivated to use the method of Oldenburg in the first place. Accordingly, the motivation to combine Oldenburg with Vandenbark, Lambert, McCoy and Mukhopadhyay is not present and thus the combination of said references under 103(a) is not tenable.

Applicants therefore respectfully submit that the obviousness rejection has been obviated and rendered moot, due to 1) the lack of motivation in combining said references; 2) the differences in said references and the method of Claim 1, and 3) the teaching away of the primary reference Oldenburg. Accordingly, Applicants respectfully request the 103(a) rejection be withdrawn and that said Claims 1-5, as herein amended, be placed into condition for allowance.

No further fee is required in connection the filing of this Amendment. If any additional fees are deemed necessary, authorization is given to charge the amount of any such fee to Deposit Account No. 08-2525.

Respectfully submitted,

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